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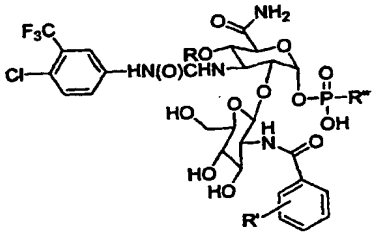
(54) Title: NOVEL DISACCHARIDE ANTIBACTERIAL AGENTS



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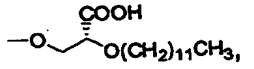

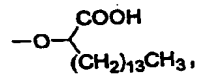

(II)

(57) Abstract: Antibacterial disaccharide and trisaccharide compounds structurally related to the moenomycin class of antibiotics in which the saccharide is covalently attached to a lipid, such as a C_4-C_{30} aliphatic alcohol, via a bridging phosphorus atom. For a non-limiting example, a compound of formula (I) in which R is hydrogen or C_1-C_4 alkyl; R' is hydrogen or trifluoromethyl; and R'' may be formula (II) or an O-linked ether of a C_4-C_{30} aliphatic alcohol. Disclosed compounds are effective as antibacterial agents, and formulations and methods of use in curing or preventing bacterial infections are disclosed.

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NOVEL DISACCHARIDE ANTIBACTERIAL AGENTS

RELATED U.S. APPLICATIONS

This application claims priority from U.S. Application 60/131,442 filed April 27, 1999.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to new disaccharide and trisaccharide compounds structurally related to the moenomycin class of antibiotics and having antibacterial activity. The compounds are disaccharides or trisaccharides covalently bonded to a lipid or lipid mimetic, such as an aliphatic alcohol, through a phosphorus linkage.

Related Background Art

The increase in bacterial resistance to conventional chemotherapy has resulted in a resurgent interest in the discovery and development of antibacterial agents. The search for novel antibiotics active against resistant phenotypes is increasingly focused on identification of novel chemotypes or antibiotics with novel mechanisms of action.

The bacterial cell wall is an attractive target for developing novel antibacterial agents. The cell wall of both gram-positive and gram-negative bacteria is essential for cell viability. Of the many enzymes involved in bacterial cell wall biosynthesis only transpeptidases responsible for crosslinking the growing glycan chain are targeted by existing clinically useful chemotherapeutic agents.

The moenomycin antibiotics are naturally-occurring phosphoglycolipids, which have been isolated from several strains of *Streptomyces*. Moenomycin A (compound **1** in **Figure 1**) is a pentasaccharide containing a long lipid attached to the reducing sugar (F) through a phosphoglycerate unit. These antibiotics have a wide range of antimicrobial activity, which is believed due to their ability to inhibit the transglycosylase activities of the bi-functional penicillin binding proteins (PBPs). These proteins catalyze the transfer of a disaccharide unit to a growing peptidoglycan chain during the biosynthesis of bacterial cell walls. To date, the moenomycins are the only known inhibitors of this enzyme activity.

The moenomycins are active against several bacterial strains, including those resistant to beta-lactam antibiotics. They are currently marketed under the tradename Flavomycine® as an additive in cattle feed, where the efficacy in promoting animal growth is believed due to

their antimicrobial activity. The moenomycins are particularly potent against gram-positive bacteria and less potent against gram-negative microbes.

The potential for moenomycin antibiotics as human therapeutics has not been studied in detail, but is expected to be limited by poor bioavailability and unfavorable pharmacodynamics. The unique mode of action and wide range of activity of this class of antibiotics makes them attractive for the study of related compounds having more favorable pharmacological properties.

At present, moenomycins, A, C_1, C_2, C_3, A_{12}, and pholipomycin compose the class of moenomycin antibiotics. Of this class, the most studied member is moenomycin A- a pentasaccharide linked to a C_{25} lipid group through a phosphate moiety. The structure of moenomycin A is shown in **Figure 1**, compound **1**. The structural similarity of this compound to the transglycosylase substrate mentioned above is readily apparent and suggests that moenomycin A acts as a competitive inhibitor of transglycosylase activity.

In the above structure, it is known that a disaccharide-phospholipid degradation produce of moenomycin A is equipotent (100% minimum inhibitory concentration (MIC) at 1 μg/mL) to the parent natural product [EP Publn. No. 130327]. In particular, it has been shown that the A, B, C and D units of moenomycin A are unimportant to the inhibition of transglycosylase activity, but that the E, F, G, H, and I groups are essential [Welzel, P., et al., (1984); Moller, U., et al., (1993); Marzian, S., et al., (1994)]. The lipid moiety I can be fully hydrogenated without substantially affecting its activity. However, in these cited studies, a free carboxylic acid function for the glyceric acid unit H appears to be necessary for inhibition. Furthermore, the structural requirements within the F-G-H region appear to be rather strict [Fehlhaber, H-W., et al., (1990); Moller, U., et al., (1993); Luning, J., et al., (1994); Heuer, M., et al., (1994)]. The structure of a fully active disaccharide degradation product of moenomycin A is shown (**Figure 1** compound **2**).

The other moenomycin compounds mentioned above differ structurally from moenomycin A principally by the number of sugar residues in the molecule and by the configuration of groups at the C4 position of the F sugar unit. In particular, only moenomycins A and A_{12} have a D sugar residue attached to the E unit. Moenomycins C_3, C_4 and pholipomycin are tetrasaccharide-phospholipids, which differ from each other by the presence or absence of hydroxyl groups at C6 positions of the C and E sugar units [Hessler-Klintz, M., et al., (1993); Scherkenbeck, J., et al., (1993)].

Moenomycin C_1 also is a tetrasaccharide and, as with moenomycin A_{12}, lacks the branching methyl group and has a change of configuration at the C4 position of the F sugar

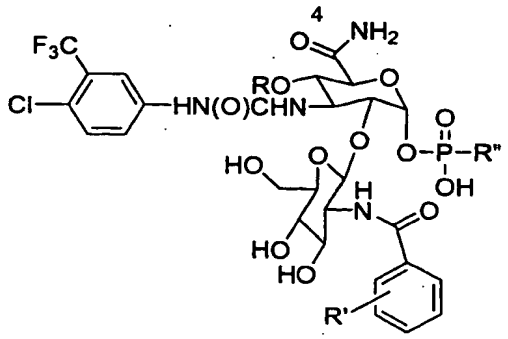
unit, i.e., the F unit is a galactopyranosiduronamide. In contrast to the other moenomycins, degradation studies of moenomycins C_1 and A_{12} reveal that the smallest antibiotically active products for these F unit galactopyranosiduronamide compounds are trisaccharide derivatives, which include the C, E and F sugar units. The additional saccharide unit (C) is required for these compounds in order to achieve the necessary hydrogen bonding environment within the active site, which is otherwise attained with a disaccharide unit in the 4-*C*-methyl glucopyranosiduronamide compounds [Hessler-Klintz, M., et al., (1993); Donnerstag, A., et al., (1995)]. In a recent series of papers, Welzel and coworkers describe the synthesis and antibiotic properties of some C-E-F trisaccharide derivatives of moenomycin A_{12} [Ritzeler, O., et al., (1997a); Ritzeler, O., et al., (1997b); Range, G., et al., (1997)]. A study of the activities of several moenomycin analogs against the *Helicobacter pylori* bacillus is the subject of EP 655249, issued to Hoechst AG.

It is desired to design and identify compounds having several of the basic structural features of the moenomycin degradation products discussed above, which retain anti-microbial activity. Of particular interest are compounds having better pharmacological properties yet retaining the broad spectrum of moenomycin activities. Of particular interest are activities against resistant strains of microorganisms. Generally speaking, it is desired to broaden the spectrum of activities and/or enhance the potencies of moenomycin antibiotics, particularly against clinically relevant microbes.

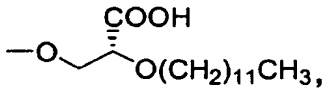
The construction of a library of disaccharides related to moenomycin A is disclosed in copending application Serial No. 08/975,229, filed November 21, 1997, which is hereby incorporated in its entirety by reference. Although the aforementioned application describes generally a library of disaccharides, it does not specifically disclose or suggest the compounds claimed in the present invention.

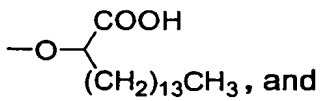
SUMMARY OF THE INVENTION

The present invention is directed to new disaccharide and trisaccharide compounds that are structurally related to the moenomycin class of antibiotics have antibacterial activity. In one embodiment, the compounds have the formula:

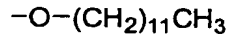


where R is hydrogen or C_1-C_4 alkyl; R' is hydrogen or trifluoromethyl; and R'' is a lipid or lipid mimetic that is covalently attached to the disaccharide or trisaccharide through a phosphoether linkage. The lipid or lipid mimetic R" is selected from:





a C_4-C_{30} aliphatic alcohol wherein the alcohol oxygen is covalently attached to phosporus. In preferred embodiments, R is hydrogen or methyl, R' is trifluoromethyl or hydrogen, and R" is:

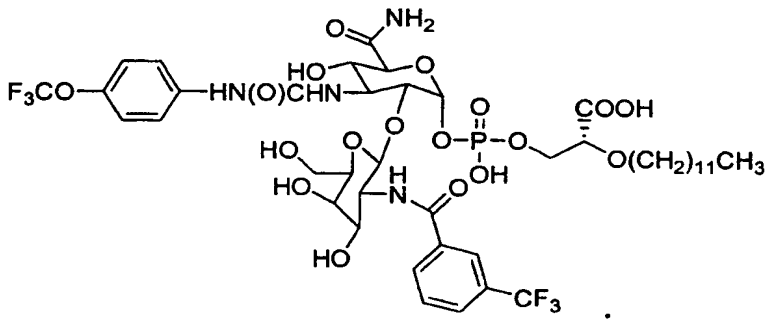


In a second embodiment, the compounds of the present invention have the general formula:

in which D represents a monosaccharide derivative or a disaccharide derivative, A represents a monosaccharide derivative, and P-R represents a C_4-C_{30} aliphatic alcohol phosphoester. In this embodiment, the anomeric carbon atom of D is covalently linked to the C2 carbon atom of A through a glycosidic linkage, and the anomeric carbon atom of A is covalently bonded to the phosphorus of P-R through an oxygen atom. In the formula, "D" represents one or more "donor" saccharide residue(s), as defined hereinafter, and is preferably a mono- or

disaccharide. The "A" group in the formula represents an "acceptor" saccharide residue, as defined hereinafter, and is a monosaccharide. Compounds represented by the above formula have a chemical structure analogous to the antibiotically active disaccharide moenomycin fragment discussed hereinabove. Accordingly, compounds represented by this formula are said to belong to a "directed" chemical library of moenomycin analogs.

A further specific embodiment of the present invention is a compound of formula:



The invention further comprises a method of treating or preventing bacterial infections in a human by administering an effective dose of one or more of the compounds of the present invention, in a pharmaceutically acceptable form, and if desired, in a pharmaceutically acceptable carrier. The invention is particularly directed to treatment or prevention of bacterial infections arising from bacteria resistant to certain other antibiotics.

BRIEF DECRIPTION OF THE FIGURES

Figure 1 Illustrates the structure of monenomycin A (structure 1), and the active disaccharide degradation product (structure 2).

Figure 2 Illustrates disaccharides immobilized upon a resin for use in solid phase synthesis of compounds of the present invention.

Figure 3 Shows acids, isocyanates, and lipids used in solid phase synthesis of the compounds of the present invention.

Figure 4 Illustrates compounds of the present invention that exhibit antibacterial activity and represent preferred embodiments.

Figure 5 Illustrates solid phase glycosylation using glycosylsulfoxide.

Figure 6 Illustrates solid phase synthesis of compounds of the present invention using a 3-O-levulinate protected sugar.

Figure 7 Illustrates solid phase synthesis of compounds of the present invention using a sugar containing an azido group at C-3.

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are used, unless otherwise described. Where specifically referred to in this application, the term "aliphatic alcohol" denotes both straight and branched-chain groups, may include unsaturated bonds, but may not include an acidic group capable of dissociating a proton. The term "C_4-C_{30} aliphatic alcohols" includes all such aliphatic alcohols having more than 3 and fewer than 31 carbon atoms, subject to the above terms. The term lipid refers generally to somewhat hydrophobic aliphatic or aromatic moietites covalently attached to the phosphate group of the compounds of the present invention. Exemplary, but non-limiting, examples of lipids that can be used in the present invention are shown in Figure 3 "Lipids". With the single exception of lipids explicitly referred to as "aliphatic alcohols," lipids that may be employed in the present invention may include additional moieties, including acidic groups capable of dissociating a proton. A phosphoether of an aliphatic alcohol denotes a covalent attachment of the –OH group of the alcohol directly between the oxygen atom of the alcohol and the phosphorus atom of the phosphate moiety. Alkyl denotes both straight and branched-chain groups. Reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to.

Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Heteroaryl encompasses a radical attached via a ring carbon of a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and $N(R_x)$ wherein R_x is absent or is hydrogen, oxo,

(C_1-C_4)alkyl, phenyl or benzyl, as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto.

It will be appreciated by those skilled in the art that compounds of the invention having one or more chiral center(s) may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis, from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine the ability of a compound to inhibit bacterial growth using the tests described herein, or using other tests which are well known in the art. The preferred absolute configuration for compounds of the invention is that shown herein.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

Specifically, (C_1 –C_4)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl. Preferred values for R' include hydrogen and trifluoromethyl.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, alpha-ketoglutarate, and alpha-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The compounds can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route

of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its slats can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper

fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pureform, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user. Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are disclosed in Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compound(s) I in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-% provided solubility permits. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%. Single dosages for injection, infusion or ingestion will generally vary between 50-1500 mg, and may be administered, i.e., 1-3 times daily, to yield levels of about 0.5-50 mg/kg, for adults.

Accordingly, the invention includes a pharmaceutical composition comprising a compound of formula I as described herinabove; or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier. Pharmaceutical compositions adapted for oral, topical or parenteral administration, comprising an amount of one or more compounds.

Resistant microorganisms are pathogenic and non-pathogenic microorganisms that are either naturally resistant to certain existing antibiotics, or that have acquired resistance as a result of exposure to antibiotics. It will be recognized by one of skill in the art that such antibiotic resistance is of increasing medical importance.

Compounds of the present invention display the greatest diversity of structural features in respect to: (i) substitutions occurring at the C3 position of residue A, (ii) substitutions occurring at the C2 position of the D saccharide, and (iii) the selection of the P-R group. Preferred substituents at the C3 position of A and the C2 position of D are amides, carbamates, ureas, sulfonamides, substituted amines, esters, carbonates, and sulfates, as described herein. Preferred P-R groups are illustrated in Figure 3.

Also contemplated is a method of preparing a library of compounds having the formula presented above. The synthesis can be performed in solution or on a support and is preferably carried out on a solid phase support.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLE 1 Library construction

A preferred synthetic approach to synthesize compounds of the present invention comprises constructing appropriately functionalized and protected disaccharide lactols that are modified at C-1, C-3, C-2', and can also be modifed in the basic structure of the disaccharide core. An advantage of this strategy is that it allows attachment of the potentially sensitive phospholipid sidechain in the last functionalization step prior to deprotection and cleavage of the product from the resin. To construct functionalized lactols on the solid phase, both direct solid phase glycosylation of resin-bound acceptor sugars followed by disaccharide

derivatization, and solid phase derivatization of disaccharides pre-constructed in solution and attached to the desired solid support, are used. Although solid phase glycosylation of acceptors **3** and **4** using glycosylsulfoxide donors **6** and **7** provides the disaccharides **8** and **9** (Figure 5) in high yield (90-95%), in other cases, complete elimination of monosaccharide byproducts is problematic. Multiple passes were not always successful at achieving high yield of the desired disaccharides. In order to maximize product purity for screening without having to execute multiple parallel purifications, the solid-phase glycosylation step is bypassed and the other target disaccharides **10** and **11**, shown in Figure 2, are preconstructed using solution chemistry. In all cases, attachment to aminoethyl-photolinker AM resin of either a monosaccharide acceptor or a disaccharide core occurs by amide bond formation through their respective C-6 carboxylate groups.

The solid phase chemistry used to build the disaccharide library is outlined in Schemes 2 and 3, and suitable reagents for library construction are shown in Figure 3. Each β-linked disaccharides utilizes a base-labile protecting group (trifluoroacetamido or phthalimido) on the C-2' amino group not only for controlling β-stereochemistry at the glycosidic linkage, but also as an easily removable group that allows for further amine derivatization. Chemical diversity at the C-3 position of the reducing sugar is explored by employing either a 3-O-levulinate protected sugar (Figure 6) or a sugar containing an azido group at C-3 (Figure 7). In the case of resin bound disaccharide **12**, the levulinate protecting group is removed under conditions that do not result in the loss of the other ester protecting groups (Figure 6), thus allowing for regioselective derivatization with isocyanates to give carbamate derivatives.

For disaccharides containing a C-3 azido group (Figure 7), regioselective derivatization is accomplished by first removing the base-labile protecting groups on the phenylthioglycoside intermediates **15** and then reacting the resulting free C-2' amino group with carboxylic acids to provide the corresponding amides. Reacetylation followed by azide reduction provided the C-3 amine ready for reaction with isocyanates or acids to give the corresponding ureas or amides.

Each disaccharide is designed to contain an anomeric thiophenyl group at the reducing terminus. This thiophenyl group acts as a masked anomeric hydroxyl. Therefore, after derivatization of the C-3 and C-2' sites is accomplished, cleavage of the anomeric thiophenyl group produces the desired lactol intermediate **13** or **16** ready for attachment of the phospholipid unit. To attach the phospholipid, phosphoramidite chemistry using modified conditions is employed to accomplish efficient solid phase oxidation which yields the desired phosphate intermediate. Following treatment under basic conditions to remove all base labile

protecting groups, photolytic cleavage provides the desired C-6 carboxamide disaccharides **14** and **17**.

A library of 1300 disaccharides is prepared using the acid, isocyanate and lipid building blocks shown in Figure 3. The library is prepared using the IRORI technology for directed sorting mix and split synthesis. Each member of the library is obtained as a discrete product. The library is screened for both inhibition of bacterial cell wall biosynthesis and inhibition of bacterial growth. The bacterial cell wall biosynthesis inhibition assay performed in a 96-well microplate format used an *E. coli* permeabilized cell membrane assay that measured incorporation of ^{14}C-*N*-acetylglucosamine into bacterial peptidoglycan. Inhibition of bacterial growth for a panel of both gram positive and gram-negative microbes is initially determined using an agar lawn assay where the compounds were applied to the agar in a microplate cover, using a 384 prong applicator and the zones of inhibition of bacterial growth are determined. Initial screening identifies compounds that inhibit both cell wall biosynthesis and bacterial growth at a screening concentration of 10 μg/mL and 25μg/mL, respectively.

General analysis of the SAR for the library showed that the three disaccharide cores **9**, **10** and **11** provide compounds with both cell wall inhibition and whole cell antibacterial activity. When combined with substitutions at C-3 and C-2', active moenomycin disaccharide analogs where the moenomycin glycerate-lipid unit were replaced with either a 2-hydroxyproprionic acid unit or a simple straight chain C-12 lipid were identified. In addition, none of the compounds containing the moenomycin-like phosphoglycerate with a C_5 or C_{22} lipid moiety demonstrated any significant potency either as a cell wall synthesis inhibitor or as an antibacterial agent. All active disaccharides contained a substituted urea at the C-3 position with a substituted aromatic urea as the preferred substituent. Unsubstituted ureas related to the natural moenomycin disaccharide C-3 substituent were shown to be inactive. None of the active compounds contained carbamates at the C-3 position. It is surprising, in light of co-pending application Serial No. 08/975,229, which defines a phospholipid as having two acidic groups, that compounds containing the simple straight chain C_{12} lipid displayed activity.

EXAMPLES 2-7: Antibacterial activities of preferred compounds.

Confirmation of the activity of the screening hits is accomplished by determining IC_{50}'s for inhibiting bacterial cell wall biosynthesis and by MIC determination using a broth dilution assay. Compounds **18** to **23**, shown in Figure 4, have IC_{50}s for inhibiting bacterial cell wall biosynthesis that are below 15 μg/mL and which have MICs below 25μg/mL. These

compounds are resynthesized and purified by HPLC chromatography. Each compound is resynthesized using the solid phase protocol described in Figure 7. In each case, crude product was obtained in approximately 30% overall yield starting with 1-4g of disaccharide functionalized resin. Purification gives 20-150 mg of pure product as the α-anomeric phosphate derivative as determined by NMR spectroscopy. The resynthesized and purified compounds are shown to have IC_{50} values for inhibition of cell wall biosynthesis of 8 to 10 μg/mL and MIC values of 3.12 to 12.5 μg/mL that are similar to those obtained from the initial screening set (see Table 1). Compounds **18** to **23** are also screened against a panel of clinically relevant sensitive and resistant gram positive bacteria and demonstrate antibacterial activity with MICs in the 4 to 128 μg/mL range.

Compared to vancomycin, compounds **18** to **23** are equipotent to vancomycin as an inhibitor of cell wall biosynthesis (Table 1). In addition, compounds **19** and **20** have comparable antibacterial activity to vancomycin (see Table 1) when evaluated against the panel of clinically relevant sensitive bacteria. Against resistant organisms, these compounds were shown to be more effective than vancomycin.

The compounds of this invention are useful in preventing or treating bacterial infections in humans. In the method of this invention, an effective amount of a compound of this invention is administered to a human.

All publications, patents, and patent documents referred to herein are hereby incorporated in their respective entireties by reference. The invention has been described with reference to the foregoing specific and preferred embodiments. However, it should be understood that many variations may be made while remaining within the spirit and scope of the invention. Therefore, the foregoing examples are not limiting, and the scope of the invention is intended to be limited only by the following claims.

Table 1. Inhibition of Bacterial Cell Wall Biosynthesis and Bacterial Growth

Compound	PGP IC_{50} (g/mL) X±SE	MIC g/mL									
		Sensitive Strains					Resistant Strains				
		E. faecalis ATCC 29212	*E. faecium* ATCC 49624	*S. aureus* ATCC 29213	*S. epi.* ATCC 12228	*E. coli* BAS849	*E. feacium* CL4931 (VanA)	*E. feacium* CL5242 (VanB)	*E. faecalis* CL4877 (Van B)	MRSA CL3033	MRCNS CL3069 (*s. epi.*)
18	6.8±0.3	12.5	12.5	12.5	12.5	25	64	128	16	32	64
19	9.2 (n=2)	3.12	3.12	6.25	6.25	>25	64	64	4	8	8
20	8.2 (n=2)	6.25	6.25	6.25	6.25	12.5	8	>128	4	4	8
21	10.6±1.1	3.12	6.25	12.5	12.5	n.t.	32	64	8	16	16
22	15.4±3.2	3.12	12.5	6.25	12.5	n.t.	128	128	128	128	128
23	9.2±3.9	6.25	6.25	6.25	6.25	>25	64	128	8	16	16
Moenomycin	0.025± 0.014	≤0.039	>200	≤0.039	≤0.039	≤0.039	2	2	1	≤0.008	≤0.008
Vancomycin	5.2±0.08	6.25	0.78	3.13	3.13	0.78	2048	32	2048	4	8

WO 00/64915 PCT/US00/11177

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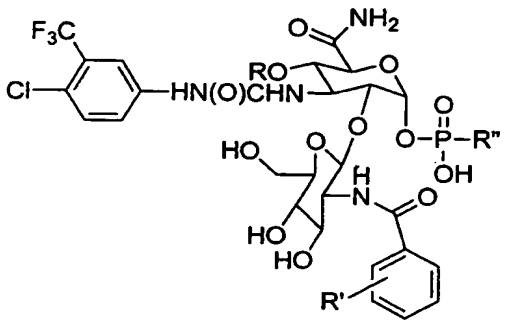
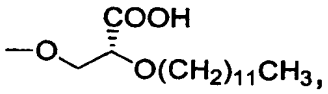
2. Moller, U., et al., Tetrahedron, 49:1635-1648 (1993)
3. Marzian, S., et al., Tetrahedron, 50:5299-5308 (1994)
4. Fehlhaber, H-W., et al., Tetrahedron, 46:1557-1568 (1990)
5. Luning, J., et al., Tetrahedron Letters, 35:1859-1862 (1994)
6. Huer, M., et al., Tetrahedron, 50:2029-2045 (1994)
7. Hessler-Klintz, M., et al., Tetrahedron, 49:7667-7678 (1993)
8. Scherkenbeck, J., et al., Tetrahedron, 49:3091-3100 (1993)
9. Donnerstag, A., et al., Tetrahedron, 51:1931-1940 (1995)
10. Ritzeler, O., et al., Tetrahedron, 53:1665-1674 (1997a)
11. Ritzeler, O., et al., Tetrahedron, 53:1675-1694 (1997b)

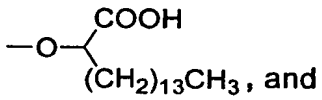
WHAT IS CLAIMED IS:

1. A compound of formula



wherein R is hydrogen or C_1-C_4 alkyl; R' is hydrogen or trifluoromethyl; and





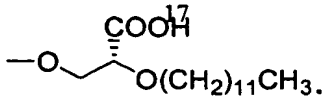
R'' is selected from the group consisting of

an O-linked ether of a C_4-C_{30} aliphatic alcohol.

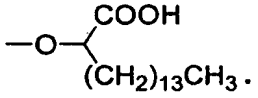
2. The compound of claim 1 in which R' is 3-trifluoromethyl or hydrogen.

3. The compound of claim 2 in which R is hydrogen or methyl.

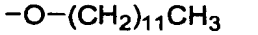
4. The compound of claim 1 in which R is methyl, R' is hydrogen, and R'' is:



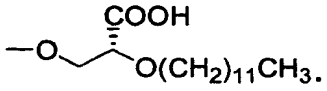
5. The compound of claim 1 in which R is methyl, R' is trifluoromethyl, and R" is:



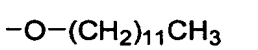
6. The compound of claim 1 in which R is methyl, R' is trifluoromethyl, and R" is:



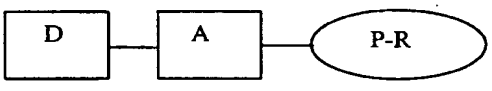
7. The compound of claim 1 in which R is hydrogen, R' is trifluoromethyl, and R" is:



8. The compound of claim 1 in which R is hydrogen, R' is trifluoromethyl, and R" is:



9. A compound having the formula:



wherein D represents a monosaccharide or disaccharide, A represents a monosaccharide, and P-R represents a phosphoether of a C_4-C_{30} aliphatic alcohol, wherein the anomeric carbon atom of D is covalently linked to the C2

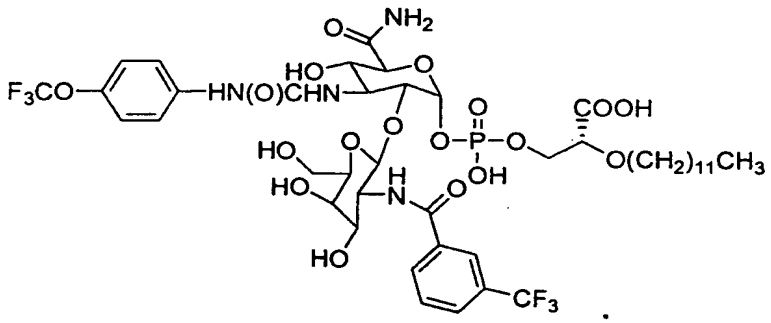
carbon atom of A through a glycosidic linkage and the anomeric carbon atom of A is covalently bonded to P-R through an oxygen atom.

10. The compound of claim 9 in which P-R represents a phosphoether of a C_8-C_{20} aliphatic alcohol.

11. The compound of claim 9 in which P-R represents a phosphoether of a C_{12}-C_{16} aliphatic alcohol.

12. The compound of claim 9 in which P-R represents a phosphoether of a C_{12} aliphatic alcohol.

13. A compound of formula:



14. A method of treating bacterial infections, said method comprising administering to a human an effective amount of the compound of claim 1, or pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

15. A method of treating bacterial infections, said method comprising administering to a human an effective amount of the compound of claim 9, or pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

16. A method of treating bacterial infections; said method comprising administering to a human an effective amount of the compound of claim 13, or pharmaceutically acceptable salt thereof in a pharmaceutically acceptable carrier

17. A method of preventing bacterial infections; said method comprising administering to a human an effective amount of the compound of claim 1, or pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

18. A method of preventing bacterial infections; said method comprising administering to a human an effective amount of the compound of claim 9, or pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier

19. A method of preventing bacterial infections; said method comprising administering to a human an effective amount of the compound of claim 13, or pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier

20. The method of claim 14 in which the bacterial infection comprises resistant microorganisms.

21. The method of claim 15 in which the bacterial infection comprises resistant microorganisms.

22. The method of claim 16 in which the bacterial infection comprises resistant microorganisms

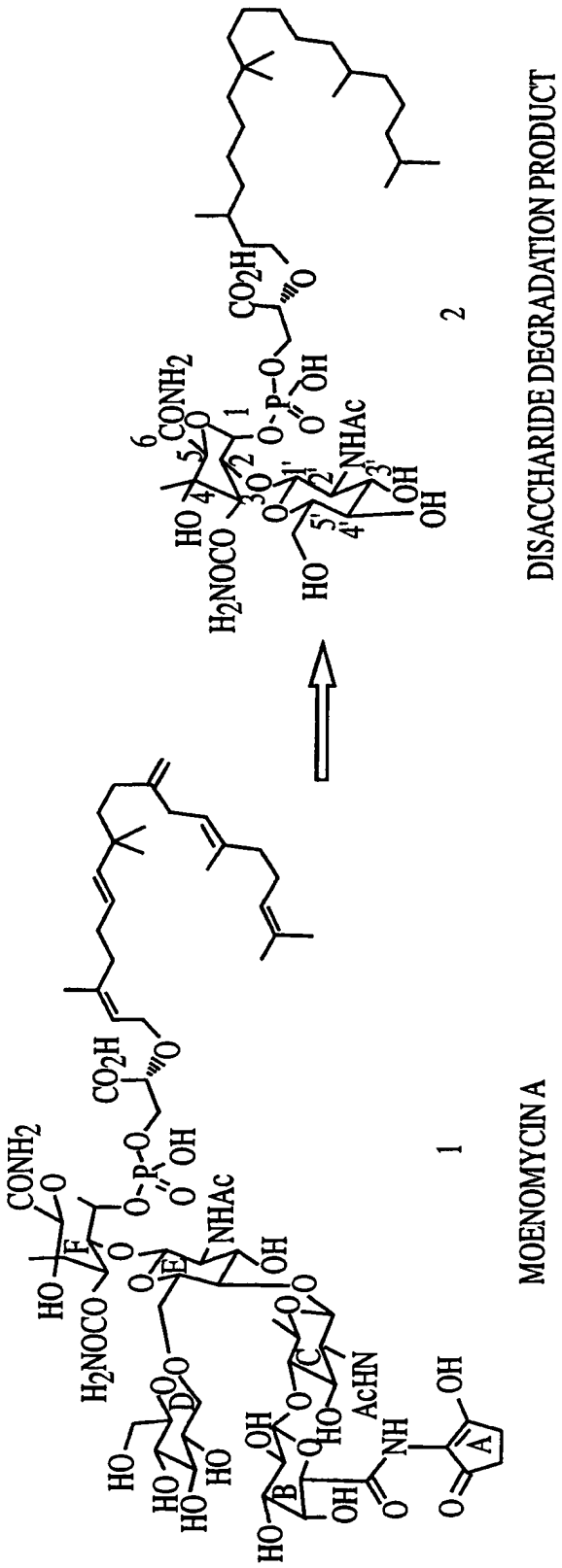
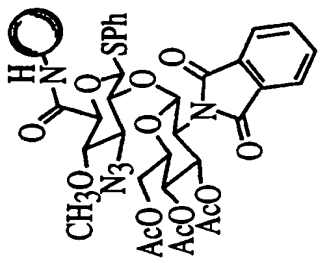
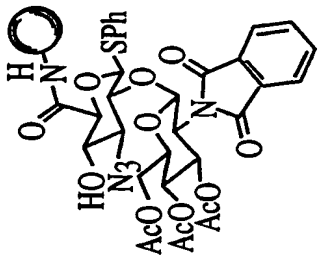


FIG. 1



11

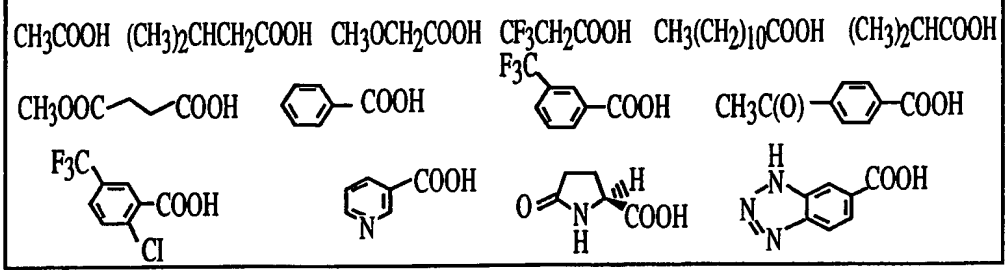


10

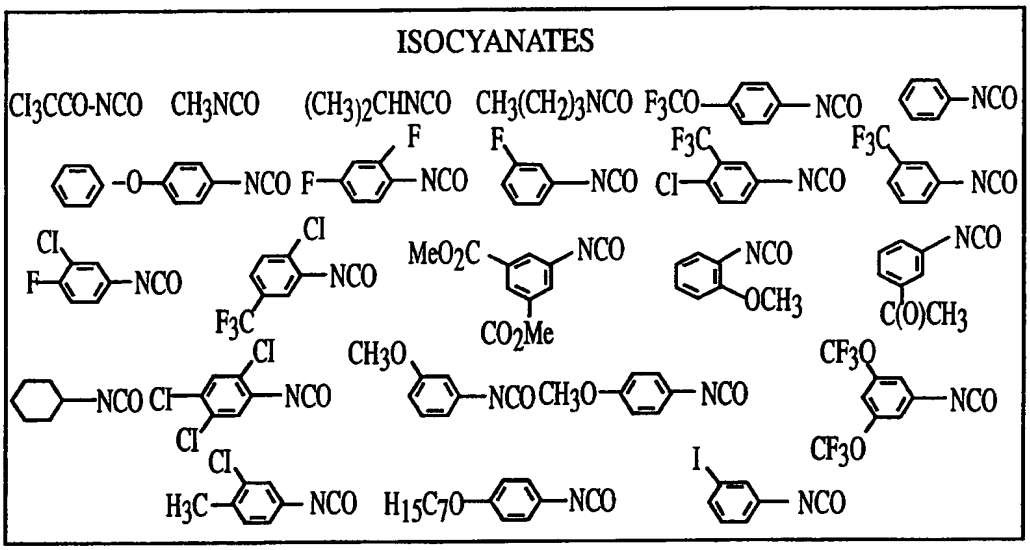
FIG. 2

LIBRARY BUILDING BLOCKS

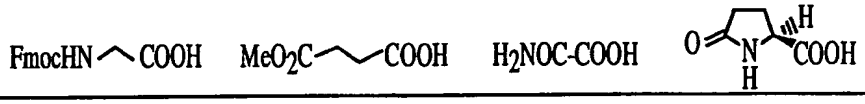
ACIDS



ISOCYANATES



ACIDS TO GENERATE AMIDES AT C-3



LIPIDS

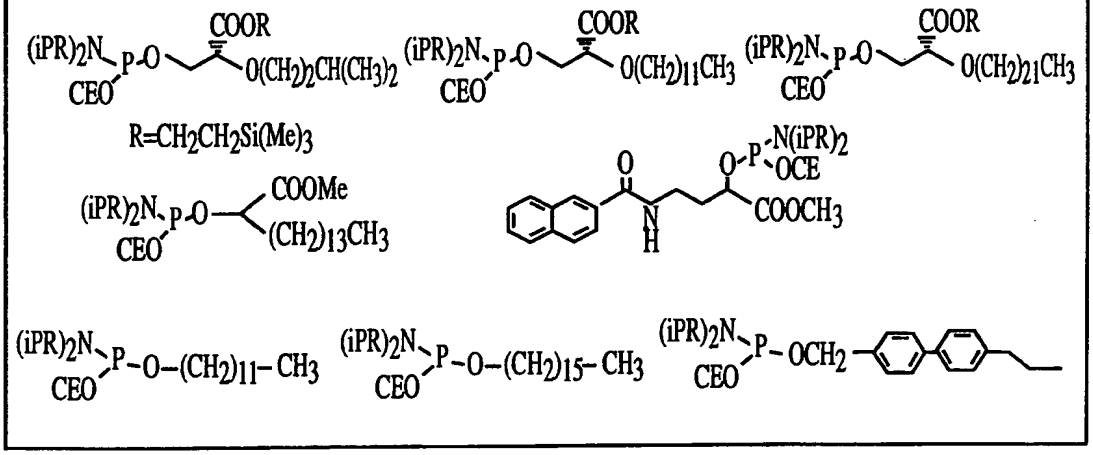


FIG. 3

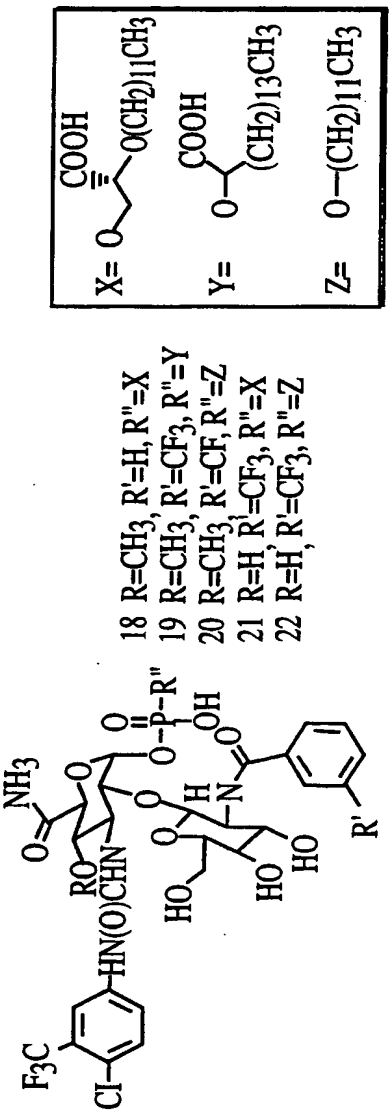

X=
Y=
Z=
18 R=CH3, R'=H, R"=X
19 R=CH3, R'=CF3, R"=Y
20 R=CH3, R'=CF, R"=Z
21 R=H, R'=CF3, R"=X
22 R=H, R'=CF3, R"=Z
23

FIG. 4

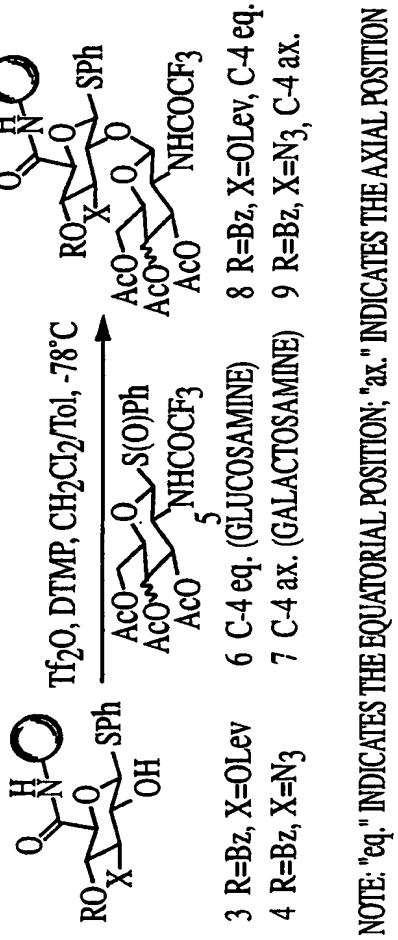
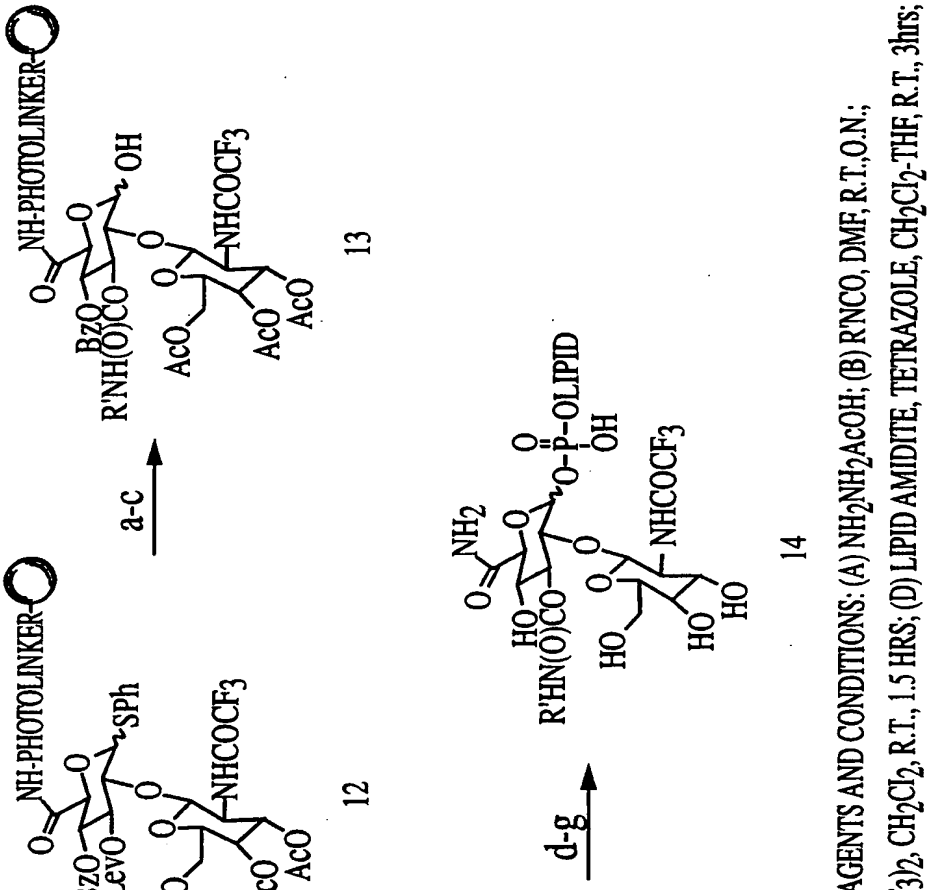
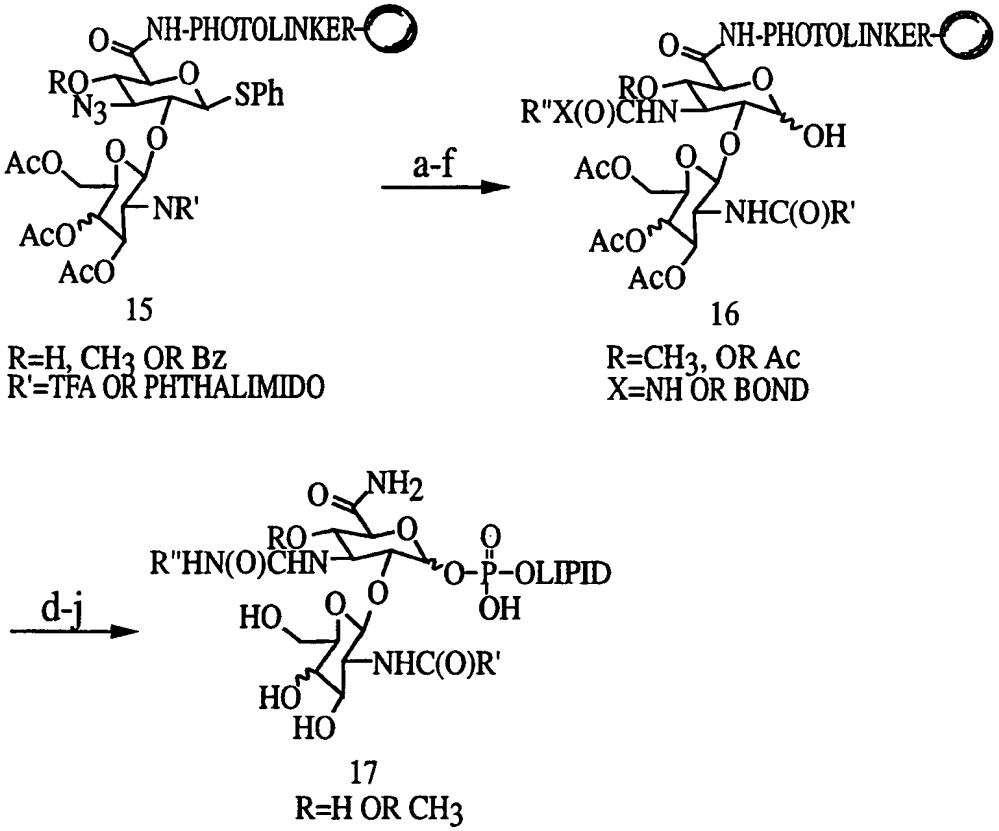

Tf2O, DTMP, CH2Cl2/Tol, -78°C
3 R=Bz, X=OLev
4 R=Bz, X=N3
6 C-4 eq. (GLUCOSAMINE)
7 C-4 ax. (GALACTOSAMINE)
8 R=Bz, X=OLev, C-4 eq.
9 R=Bz, X=N3, C-4 ax.
NOTE: "eq." INDICATES THE EQUATORIAL POSITION; "ax." INDICATES THE AXIAL POSITION

FIG. 5



[a] REAGENTS AND CONDITIONS: (A) NH_2NH_2AcOH; (B) R'NCO, DMF, R.T.,O.N.; (C) $Hg(OCOCF_3)_2$, CH_2Cl_2, R.T., 1.5 HRS; (D) LIPID AMIDITE, TETRAZOLE, CH_2Cl_2-THF, R.T., 3hrs; (E)O_2, THF, R.T.; (F) 0.1M $LiOH{\cdot}H_2O$/ THF-MeOH(4:1), R.T., 1HR; (G) HU_{365nm}, THF, O.N..

FIG. 6



[a] REAGENTS AND CONDITIONS: (A) 1M hydrazine/THF OR 0.5M LiOH/THF-MeOH(1:1), R.T.,O.N.; (B)R'CO_2H, HATU,DIPEA, DMF, R.T.,O.N.; (C) Ac_2O, DMAP, CH_2Cl_2, R.T.,O.N.; (D)Me_3P, H_2O, THF-EtOH(1:1), R.T., 2hrs; (E)R"NCO, DMF, R.T., 4hrs; OR R"CO2H, HATU, DMF, R.T. (F) $Hg(OCOCF_3)_2$, CH2, R.T., 1.5HRS; (G) LIPID AMIDITE, TETZEROLE, CH_2Cl_2-THF(1:1), R.T., 3HRS; (H) *m*CPBA, CH_2Cl_2, R.T., 30MIN; (I) 0.1M LiOH/THF-H_2O-MeOH(7:2:1), R.T., 1HR; (J) $h\upsilon_{365NM}$, THF, O.N..

FIG. 7

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/US 00/11177

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H11/04 A61K31/70 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 684 626 A (WELZEL PETER ET AL) 4 August 1987 (1987-08-04) abstract	1,14
X	H. HOHGARDT ET AL.: "Synthesis of two structural analogues of the smallest antibiotically active degradation product of moenomycin A" TETRAHEDRON, vol. 44, no. 18, 1988, pages 5771-5790, XP002142176 page 5775, structure 14b	9
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2000	09/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	de Nooy, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat' Application No
PCT/US 00/11177

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	M.J. SOFIA ET AL.: "Discovery of novel disaccharide antibacterial agents using a combinatorial library approach" JOURNAL OF MEDICINAL CHEMISTRY, vol. 42, no. 17, 1999, pages 3193-3198, XP002142177 the whole document	1-22

2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/11177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4684626 A	04-08-1987	DE 3318594 A	22-11-1984
		DE 3467171 D	10-12-1987
		EP 0130327 A	09-01-1985
		JP 59222499 A	14-12-1984